EPR-based pH mapping with a method of partially scanned spectral-spatial imaging

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Introduction

One of the key biological parameters in the maintenance of physiological homeostasis is pH. Deviation from physiological normal values of pH is typically observed in a number of pathological conditions such as cancer, cardiac infarction and brain infarction, which result in tissue ischemia and acidosis due to lowered pH. As such, a shift in pH can be used as a marker of the severity or progression of a particular physiological condition. 31P-NMR has proven to be the most suitable approach for noninvasive pH detection. However, pH assessment using 31P-NMR and inorganic phosphate, Pi, has its own limitations, including lack of resolution, the fact that Pi concentrations vary with metabolism and ischemia, and its chemical shift depends on ionic strength. Therefore, there is a strong interest towards development of EPR-based approaches for pH detection due to potential for higher functional resolution [1,2] In this study, we introduce a newly developed method for pH measurement using continuous-wave (CW) EPR spectroscopy, with improved spatial and functional resolution, and present preliminary results of pH-imaging of phantoms using pH-sensitive nitroxide probe.

Methods

EPR imaging is based on the measurement of EPR spectra of free radicals, and in the case of this study, the pH sensitive free radical molecule 4-amino-2,2,5,5-tetramethyl-3-imidazoline-1-yloxy, has been used [1] (Fig. 1). The distance between spectra, known as the hyperfine coupling (hfc), is altered by pH of the local environment (Fig. 2). By acquiring hfc measurements across a range of known pH samples, a calibration curve (hfc vs known pH) can be constructed (Fig. 3). For calibration curve measurements 1mM spin probe solution in phosphate buffer was prepared. Precision of the measured hfc values was checked by repeating the measurements 10 times. Additionally, to obtain spatial as well as spectral imaging information, the EPR technique of three-dimensional spectral-spatial imaging can be used. In this study, we introduce a method for obtaining pH measurements using two components of EPR spectra thereby reducing the missing angle and field of view. Using a low magnetic field component, we firstly reconstructed a spectral-spatial image. This process was repeated for the center field component, thereby providing two spectral-spatial images, which are superimposed and subtracted to give a difference in spectral position equivalent to the hfc value. The measured hfc value can then be interpreted as a pH value, using the previously determined hfc/pH calibration curve.

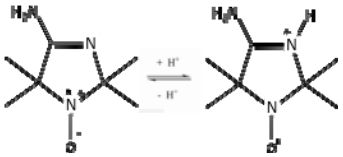

Fig. 1. 4-amino-2,2,5,5-tetramethyl-3-imidazoline-1-yloxy spin probe structure

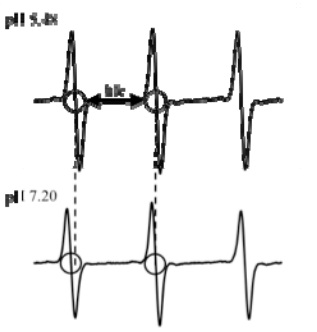

Fig. 2. EPR spectra of pH probe. The hfc value measured as distance between low- and center-field spectral components depends on pH (see Fig. 3).

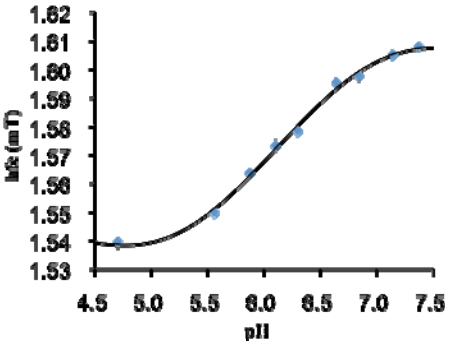

Fig. 3. pH vs hfc calibration curve. hfc values shows mean measured values ± S.D.

Results

We used four tubes of known pH, and spin probe concentration of 1 mM/0.5 ml (Fig. 4). Figure 5(a) shows pH distribution maps and mean measured pH values ± 1 S.D., and Fig. 5(b) the corresponding number of pixels vs pH for each tube. The images show good homogeneity with mean pH values comparing favorably with the true pH values, shown in Fig. 4.

Discussion and Conclusion

In this study, we demonstrated a new method for pH imaging with CW-EPR. From preliminary phantom measurements, we obtained pH values with a narrow distribution of values (± 0.05 pH), with an average accuracy across four different values of <0.04 pH. In future work, we explore the use of this technique in *in vivo* studies of mice.

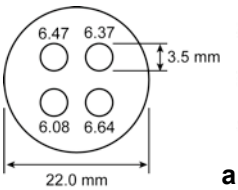

Fig. 4. pH phantom

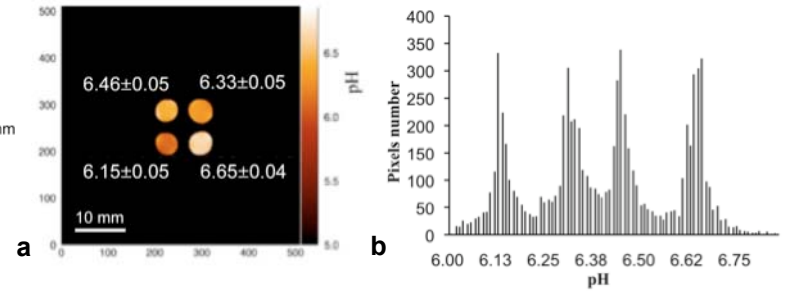

Fig. 5. (a) pH mapping of the phantom and (b) its histogram pH values.

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References

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